Form, Storage and Usage: Blots are provided pre-blocked and in ready-to-use forms. Store unused blots at 4°C in a sealed bag. These blots should be used within 3-4 months.

Prior to its use, the ReadyBlot should be immersed in 100% methanol for 10 seconds followed by washing with PBS twice to remove residual methanol. Finally, the wet blot should be transferred into the desired antibody solution.

Recommended Usage

These blots will be most useful for proteins that are relatively abundant in whole brain tissue. Very low abundant proteins that require the use of enriched cell membranes or nuclear fractions may be poorly represented in whole tissue blots. An attempt has been made to equalize the protein load with beta-actin. However, antibody reactivity with beta-actin in various regions may differ due to selective posttranslational modifications) or the fact that the antibody may not react with certain actin-isoforms (e.g., muscle). It is also important to realize that there is NO protein that remains the same in ALL physiological or pathological conditions. But beta-actin, tubulin, or glyceraldehydes-3-phosphate dehydrogenase has often been used as controls. Therefore, we recommend that the researchers compare the intensity of beta-signal with the intensity of the target protein in actual blot and conditions used. Beta-actin antibody is also available.

Re-use: It is possible to re-use the blot by stripping the antibodies with the Western blot recycling kit (cat # 90100) immediately after probing and recording results. The stripped and re-blocked blots can be used immediately or stored for later use. See details of stripping kit at our web site.

Ordering Information

ReadyBlot **Newborn Mouse Brain** Protein Explorer, Cat # MBWB-12; $495
ReadyBlot **Adult Mouse Brain** Protein Explorer, Cat # MBWB-11; $495
ReadyBlot Brain **Old Mouse Brain** Protein Explorer, Cat # MBWB-13; $595

Rat/Mouse ReadyBlot **Brain** (12 regions) Protein Explorer
Rat/Mouse ReadyBlot **Tissue** (10 major tissues) Protein Explorer
Rat/Mouse ReadyBlot **Kidney** (8 major regions) Protein Explorer
Rat/Mouse ReadyBlot **Digestive Tract** (12 regions) Protein Explorer

Please see details at our web site.

Related Products:

1. Mouse monoclonal **beta-actin antibody**, cat # ACTB12-M; $245/100 ug

2. **Western blot recycling kit** (strip antibodies in ~15 min. at room temp and re-use blots; sufficient reagents to strip 20-40 mini blots), Cat # 90100, $195.

3. **Western blot kit** (contains all necessary blocking, wash, antibody dilute, ECL reagents and a specified (anti-rabbit, mouse etc antibody conjugates; sufficient for 15-30 blots), Cat # 80200, $295 per kit.

Instruction Manual No. MBWB-12

ReadyBlot New Born Mouse Brain Protein Explorer

Cat. No. MBWB-12

Study distribution of proteins in 12 major regions of mouse brain with premade protein blots

ALPHA DIAGNOSTIC INTERNATIONAL

India Contact:

Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034
Ph: +91-11-42208000, 42208111, 42208222

Mobile: +91-9810521400
Fax: +91-11-42208444
Email: customerservice@atzlabs.com
Web: www.atzlabs.com

LIFE TECH Life Technologies™ Your Molecular & Cell Technology Partner

DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

ReadyBlot **New Born Mouse Brain** Protein Explorer
Cat # MBWB-12

Study distribution of Proteins in Rat Brain in Hours!

Brain is one of the most complex organ in higher vertebrates. It is responsible for controlling growth, physical, mental, physiological, endocrinological, and emotional activities. The brain cells are inherently complex and obviously depends upon the ability of its cells to specialize in one or more of these activities. Brain tissues have been divided into dozens of major and hundreds of sub-areas that are anatomically and functionally distinct. The brain cells achieve these specialized activities by expressing hundreds of specific house keeping proteins, enzymes, transporters, and receptors. Expression of many genes in various areas of the brain is age and development related and may undergo an irreversible change. Therefore, it is very important to study the normal and abnormal expression of various proteins in order to delineate their physiological functions.

Acquisition of animal or human brain tissue is not only time-consuming and expensive, but also requires expertise and training in brain anatomy, cell and molecular biology. ADI has carefully dissected and processed 12 anatomically and functionally distinct areas of rat brain for the study of proteins using Western blots. The brain proteins have been electrophoresed, electro-blotted, and blocked. A lane of pre-stained mol. wt markers is included in each blot to assist you in identifying the size of the proteins.

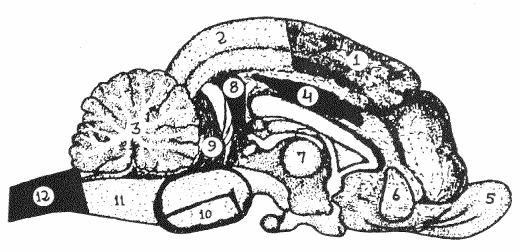
Animals: Normal Adult Mouse (Balb/c, Male, Av. Weight ~25-35 g).

Brain Tissue Extraction: Whole Brain tissue was collected after quick decapitation and washed in cold normal saline to remove any contaminating blood. Various regions of the brain, as outlined in Fig. 1, were immediately dissected and kept in cold extraction buffer [50 mM Tris, pH 6.8, 1 mM EDTA, 2% SDS, 1 ug/ml each of protease inhibitors (leupeptin, aprotinin, and pepstatin)]. Protein concentration was equalized in samples from all brain regions.

SDS-Gel Electrophoresis and blotting: Brain tissue extracts were mixed with 2X standard Laemmeli **reducing** buffer, heated for 5 min. at 90^{o}C. Brain tissue proteins (~25-30 ug) were run on 4-20%-reducing SDS-mini gels at 125 V for approx. 90 min. Pre-stained mol. Wt markers (Biorad # 161-0324) were:

Label	Marker	Color	Size KDa
A	Myosin	Blue	199
B	beta-galactosidase	Magenta	128
C	Bovine serum albumin	Green	85
D	Carbonic anhydrase	Violet	41.7
E	Soybean trypsin inhibitor	Orange	32.1
F	Lysozyme	Red	18.3
G	Aprotinin	Blue	7.5

Fig 1. Various Regions of Brain used in the blot



1. Frontal Cortex
2. Posterior Cortex
3. Cerebellum
4. Hippocampus
5. Olfactory bulb
6. Striatum
7. Thalamus
8. Midbrain
9. Entorhinal Cortex
10. Pons
11. Medulla
12. Spinal Cord

SDS-Gel Electrophoresis and blotting

The proteins were transferred to **PVDF** (0.45μm) and the homogeneity of protein transfer in lanes (1-12) was verified using water soluble Stain-ALL (ADI Cat # SALL-500). Equal amount of protein in each lane was also verified using anti-beta-actin antibody as a probe (Fig. 2). Pre-stained mol. Wt standards have been marked A-G on the blot. Top (T) and bottom (B) of the gel/blot are also marked to identify the boundaries.
Proteins (Lanes 1-12) in the extracts of different regions (1-12 in Fig 1) of brainwere stained with comassie (Fig 2) probed anti-beta-actin antibody (Fig 3).

Blocking: After destaining, PVDF membranes were blocked with 1:10 diluted PBS/milk-based buffer (ADI Cat# 80062) and dried using proprietary techniques to minimize damage to the membrane and proteins.